



# The hitchhiker's guide to Australia: the 18,000-km-long journey of *Alepis viatrix* Jaume-Schinkel, Kvifte, Weele & Mengual, 2022 (Diptera, Psychodidae) discovered through citizen science

SANTIAGO JAUME-SCHINKEL<sup>1\*</sup>, XIMO MENGUAL<sup>1</sup>, ANDY G. HOWE<sup>2</sup>, ERINN P. FAGAN-JEFFRIES<sup>3</sup>

<sup>1</sup> Museum Koenig, Leibniz-Institut zur Analyse des Biodiversitätswandels, Bonn, Germany • SJS: s.jaume@leibniz-lib.de  <https://orcid.org/0000-0002-3502-9407> • XM: x.mengual@leibniz-lib.de  <https://orcid.org/0000-0002-6185-9404>

<sup>2</sup> Forest Research Institute, University of the Sunshine Coast, Sippy Downs, Australia • ahowe@usc.edu.au  <https://orcid.org/0000-0002-7460-5227>

<sup>3</sup> School of Biological Sciences, The University of Adelaide, Adelaide, Australia • erinn.fagan-jeffries@adelaide.edu.au  <https://orcid.org/0000-0002-3322-6255>

\* Corresponding author

**Abstract.** The Neotropical genus *Alepis* Enderlein, 1937 (Diptera, Psychodidae) is newly recorded in Australia. We present new geographical records for *Alepis viatrix* Jaume-Schinkel, Kvifte, Weele & Mengual, 2022, extending the range of this species by 18,000 km. We attribute these new Australian records to the likely unintentional introduction of *A. viatrix* through international bromeliad trade. This moth fly was found by school children working with insect taxonomists through an Australian citizen-science project, Insect Investigators. We describe and present for the first time high-resolution SEM pictures of the eggs of the genus *Alepis*.

**Keywords.** Moth flies, introduced species, dark taxa, DNA barcoding, taxonomy, new distribution, new record, community science, school project

Academic editor: Alessandre Pereira-Colavite

Received 5 June 2023, accepted 22 August 2023, published 30 August 2023

Jaume-Schinkel S, Mengual X, Howe AG, Fagan-Jeffries EP (2023) The hitchhiker's guide to Australia: the 18,000-km-long journey of *Alepis viatrix* Jaume-Schinkel, Kvifte, Weele & Mengual, 2022 (Diptera, Psychodidae) discovered through citizen science. Check List 19 (4): 589–597. <https://doi.org/10.15560/19.4.589>

## Introduction

Introduced species, also known as non-native, casual, or alien species, overcome geographical barriers in their native range, increasingly as an unintentional consequence of human-mediated international trade and travel pathways (Lockwood et al. 2005; Minchin 2009; Simberloff et al. 2013). Upon reaching new localities, an introduced species must successfully reproduce to become established at which point it may be considered naturalized. Only when a species has detrimental impacts on at least one aspect of the environment, human health, or economy is it considered “invasive” (Blackburn et al. 2011). Invasive species are key drivers of global declines in biodiversity (Simberloff et al. 2013; Wagner 2020). Furthermore, introduced species

usually exist unnoticed by the scientific community during the establishment phase, especially during the initial period of low abundance (Encarnaçao et al. 2021), and insect species that successfully mount incursions are often not intercepted at international borders (Caley et al. 2015; Turner et al. 2021). One of the most effective options to detect introduced species is through citizen science (Encarnaçao et al. 2021), which is defined as “active involvement of citizens in scientific inquiry generating new knowledge or understanding” (Wiggins and Crowston 2011). The involvement of the general public in scientific research allows early monitoring and tracking of species’ dispersal over a large geographical area at a relatively low cost (Eitzel et al. 2017; Encarnaçao et al. 2021; Epanchin-Niell et al. 2021) and simultaneously benefits participants by increasing

environmental awareness (e.g. Schreck-Reis et al. 2013; Payne et al. 2023).

Invasive species can be found among Diptera, the two-winged insect order that comprises gnats, midges, mosquitos, and true flies. The natural history of dipterans is very diverse and include predation, phytophagy, parasitism, and saprophagy among the common feeding strategies. Taxa with aquatic or semi-aquatic larvae can develop in artificial water bodies, such as canals, reservoirs, dams, or containers, and they can be unintentionally spread by human activities. There are well-documented examples of invasive dipterans that breed in containers, such as mosquitoes and moth flies (Reiter 1998; Zielke et al. 2015; Kvifte 2023).

Among the moth flies (Insecta, Diptera, Psychodidae), *Alepis* Enderlein, 1937 is considered one of the most diverse Neotropical genera with 60 described species, the majority of which are naturally distributed in the Neotropics (Tonnoir 1920; Quate 1963, 1999; Duckhouse 1974; Wagner 1993; Bravo et al. 2004; Quate and Brown 2004; Wagner and Hibbar 2004; Wagner and Svensson 2006; Bravo 2008; Wagner et al. 2008, 2010; Jezek et al. 2011; Omad and Rossi 2012; Cordeiro et al. 2015, 2021; Tkoč et al. 2017; Duran-Luz et al. 2018; Jaume-Schinkel et al. 2022). To date, three species have been described beyond the expected Neotropical distributional range of the genus, and are henceforth considered as introduced species, namely, *Alepis symmetrica* Wagner & Hibbar, 2004, which was described based on specimens collected in Florida, USA and reared from a bromeliad plant; *Alepis vaga* Wagner & Svensson, 2006, described from specimens reared from a bromeliad in Sweden; and *Alepis viatrix* Jaume-Schinkel, Kvifte, Weele & Mengual, 2022, described based on specimens collected in a botanical garden on the Azores (Portugal).

In the present study, we report new *Alepis* records from Australia originating from an Australian citizen-science project, Insect Investigators. In addition, we describe and present for the first time high-resolution SEM pictures of the eggs of the genus *Alepis*.

## Methods

The examined material used for the study was collected using a Malaise trap, as part of the Insect Investigators project (<https://insectinvestigators.com.au/>). Insect Investigators partnered with 50 schools in the Australian states of Queensland, South Australia, and Western Australia, with each school monitoring a Malaise trap on or near school grounds for four weeks in March 2022. Schools sent their weekly trap samples to the University of Adelaide where samples were sorted to order level, prior to the selection of 285 specimens for DNA barcoding.

Specimens were collected into 80% propylene glycol as an initial preservative medium and transferred into 100% ethanol within a few weeks of collection. DNA extraction and sequencing of the standard 5'-end of the cytochrome c oxidase subunit I (COI), also known as

DNA barcode (Hebert et al. 2003), were performed at the Canadian Centre for DNA Barcoding at the University of Guelph, Canada, following the in-house protocols. Data were uploaded to the open-access Barcode of Life Database (BOLD; <https://www.boldsystems.org/>). After DNA extraction, specimens were preserved in ethanol. Morphological characters were observed using a Zeiss Scope A1. Specimens are deposited in the Queensland Museum (QM), Brisbane, Australia, and in Museum Koenig (previously known as Zoologisches Forschungsmuseum Alexander Koenig) (ZFMK), Bonn, Germany.

As an extra exercise, we asked the young citizen scientists (students of year 4, now year 5) to draw and write their interpretation of insect adaptation to their environment focusing on our results (available as Supplementary file, Figs S1, S2).

**Terminology.** Egg poles follow the definition of Dutra et al. (2011). The anterior pole is defined as the end of the egg that bears the pedicel or a projection, on the contrary, the posterior pole is usually round and smooth and lacks external structures or openings. Additionally, we follow the recategorization of the exochorionic sculpture patterns and terminology of de Almeida et al. (2004).

## Results

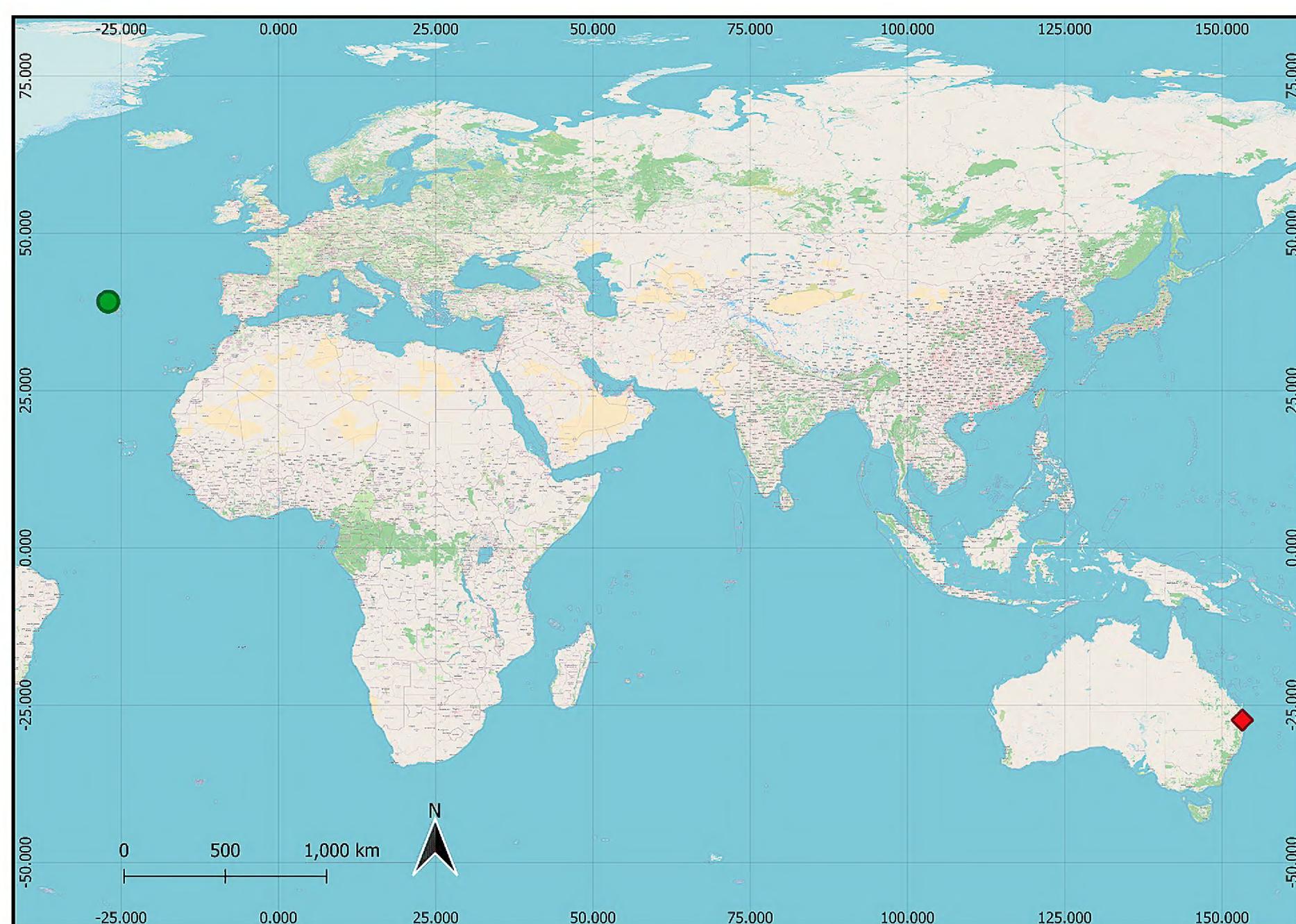
### *Alepis viatrix* Jaume-Schinkel, Kvifte, Weele & Mengual, 2022

*Alepis viatrix* Jaume-Schinkel et al. 2022: 385. Type locality: Portugal, Azores Archipelago, Terceira Island, botanical garden.

Figures 1–3

**New records** (Fig. 1). AUSTRALIA – QUEENSLAND • Brisbane, Yeronga State School; –27.519, 153.022; 8–15.III.2022; Yeronga State School students leg.; Insect Investigators week 1, Malaise trap, 1–8.III.2022; BOLD accession number ASMII2842-22, 1 ♀ (QM T259470) • same locality and collector; 8–15.III.2022; Insect Investigators week 2, Malaise trap, 8–15.III.2022; BOLD accession number ASMII2894-22, 1 ♀ (QM T259471).

**Identification.** A preliminary identification of the collected specimens was firstly based on blasting the DNA COI barcodes in BOLD. The COI sequence of the two female Australian specimens are very similar to each other, with a difference of only 0.46%. Moreover, both sequences are also quite similar to previously published sequences of *A. viatrix* with a difference of 0.05–5.22%. COI sequences can be accessed in BOLD under the Dataset DS-DTALEPIA (<https://doi.org/10.5883/ds-dtalepia>). Furthermore, the identification was supported comparing external morphology and female genitalia of the Australian specimens with the type series and, additionally, by comparison with the original description and detailed figures published by Jaume-Schinkel et al. (2022). No morphological differences were noticed between the Australian specimens and the type series individuals.



**Figure 1.** Records of *Alepis viatrix*. Green circle indicates the type locality of the species; red rhombus indicates our new record from Australia. The map was built using QGIS v. 3.28.6 with the Quick-MapServices function.

**Remarks.** Australian female individuals are morphologically and genetically congruent with the type series specimens of *A. viatrix* (see Discussion). Nevertheless, the diagnostic characteristics to distinguish *Alepis* species are all found in the male genitalia (see Jaume-Schinkel et al. 2022), as the female sex is not known for all the described *Alepis* species, especially for the morphologically closely related taxa to *A. viatrix*. This knowledge gap, together with the lack of DNA barcodes for any *Alepis* species except for *A. viatrix*, limits our determination. Although the likelihood is difficult to assess, we cannot rule out that females of other *Alepis* species share external and internal morphology with *A. viatrix*, as well as their COI sequence.

**Egg description.** Length  $0.436 \pm 0.018$  mm. Width  $0.104 \pm 0.036$  mm ( $n = 15$ ) (Fig. 2A–E). The exochorion sculptures form a distinct polygonal pattern, forming elongated hexagons running transversal along the long axis of the egg (Fig. 2A–C). The basal lamina inside the hexagons is generally smooth, with some irregularly distributed protuberances. No aeropiles were observed in the posterior pole (Fig. 2D). The anterior pole presents a conical projection of about 0.015 mm, with the apex rounded, without exochorion sculptures (Fig. 2E).

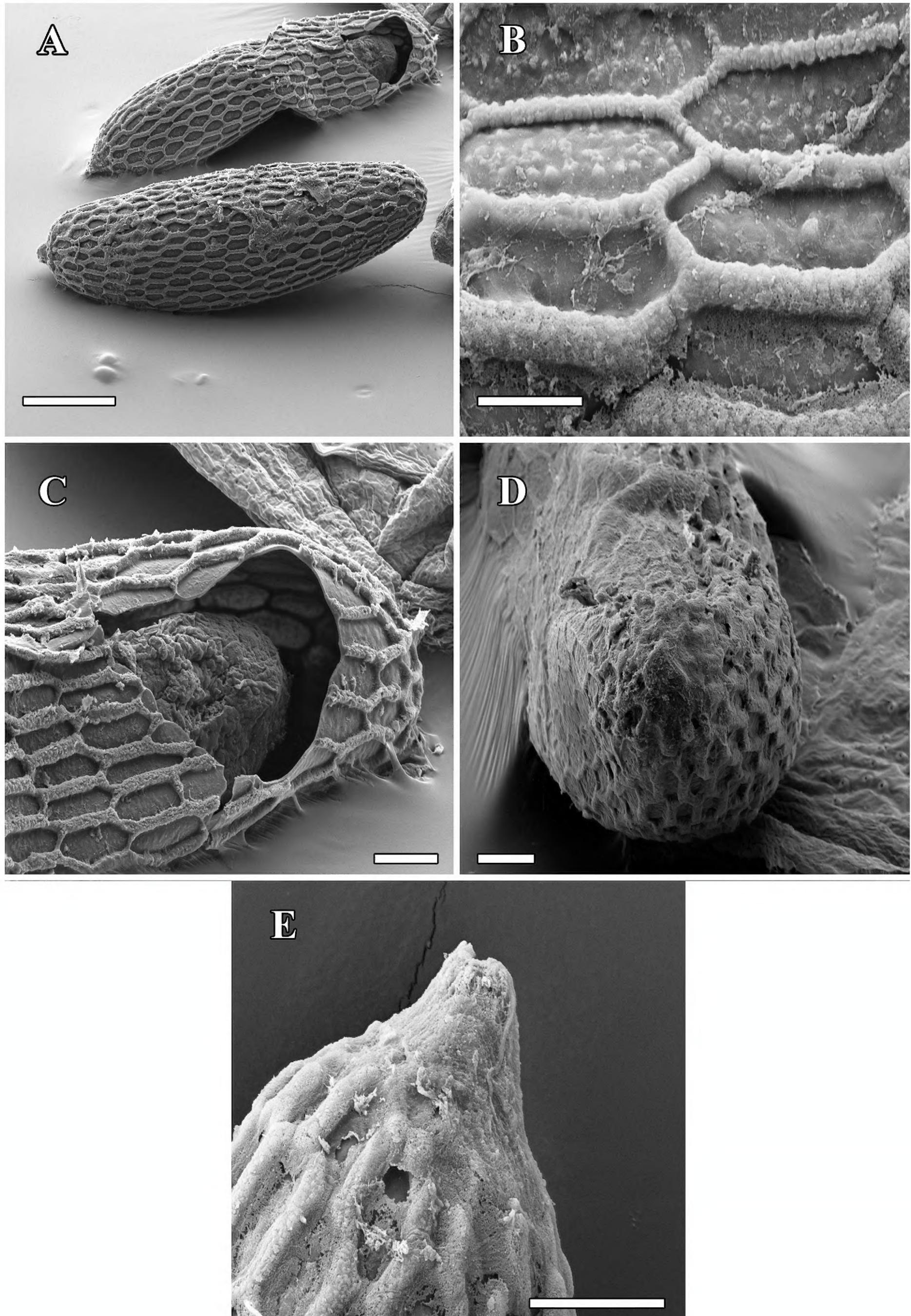
**Genital chamber.** The genital chamber is a complicated three-dimensional structure, usually portrayed in two dimensions because of the common process of preparing permanent microscope slides as the most accepted

way to study morphology in Psychodidae. The original description of the genital chamber by Jaume-Schinkel et al. (2022: figs 12–15) can be better understood while observing Figure 3A–C. With the aid of an SEM microscope, lateral (Fig. 3A) and dorsal (Fig. 3B, C) views of the genital chamber can be observed. However, the genital chamber is surrounded by membranous tissue, which hinders the observation of the internal structures; thus, only the outer structure is discernible.

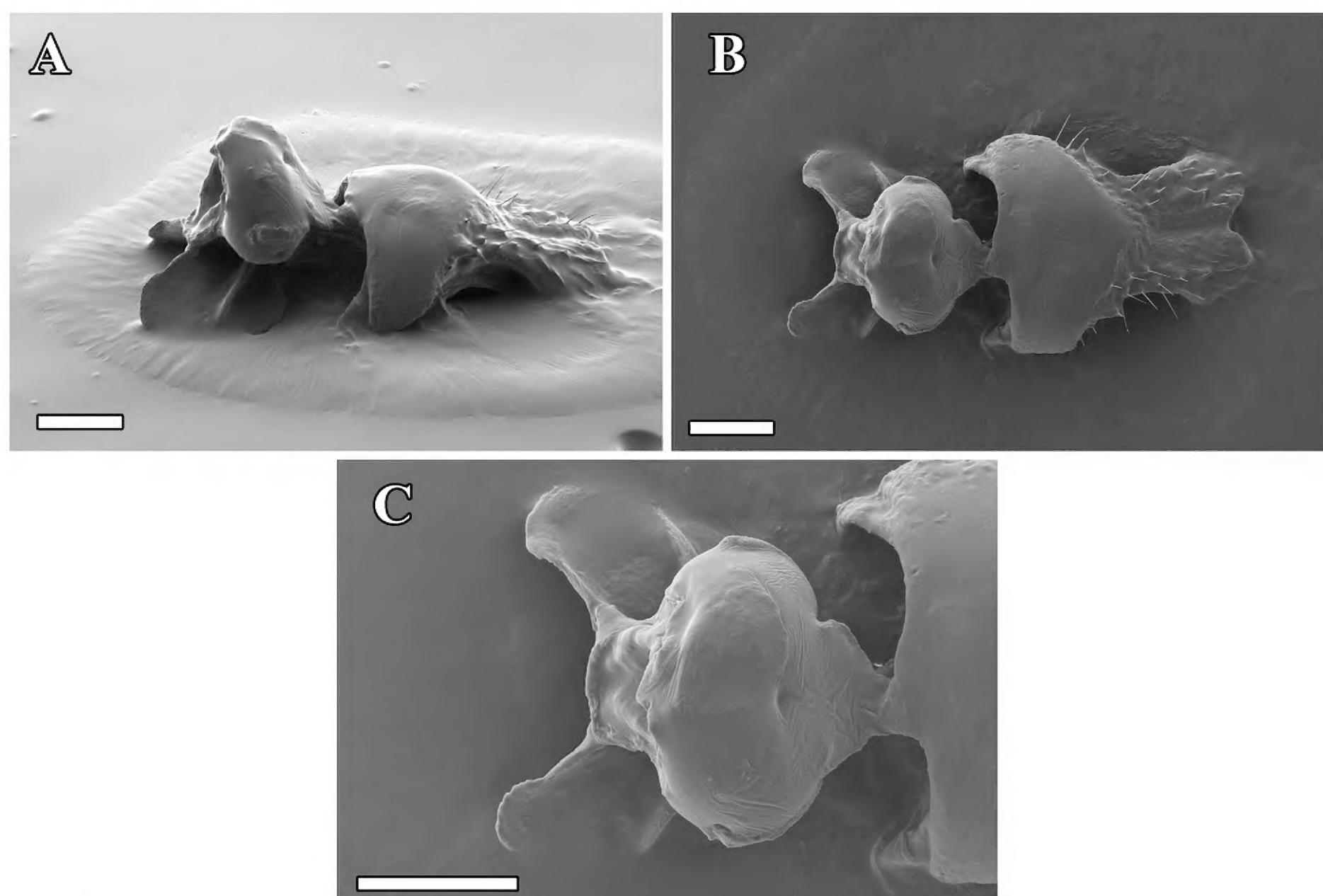
## Discussion

Our findings have greatly expanded the known distribution range of *Alepis viatrix* (Psychodidae, Psychodinae) by over 18,000 km (Fig. 1) and suggest international trading of bromeliads as the origin for the unintentional introduction of this Neotropical genus in other regions. We have discovered a new population in Australia, Queensland ( $-27.519$ ,  $153.022$ ), which is a considerable distance from the species' type locality in the Azores ( $38.6526$ ,  $-27.2190$ ). These new observations are particularly noteworthy as they constitute the first documented occurrence of the *Alepis* genus in Australia and the entire Australasian Region.

Almost nothing is known about the biology of the adults and little is known about the immature stages of the genus *Alepis* aside from the species reared from bromeliads and the reported larvae of *A. longinoi* Quate & Brown, 2004 collected in ant colonies of the genus



**Figure 2.** Eggs of *Alepia viatrix*. **A.** Lateral view. **B.** Exochorion sculptures forming elongated hexagons and showing protuberances in the basal lamina. **C.** Inside of the egg. **D.** Posterior pole of the egg. **E.** Anterior pole of the egg. Scale bars: A = 100 µm, B = 10 µm, C-E = 20 µm.



**Figure 3.** *Alepis viatrix*. **A.** Lateral view of genital chamber and subgenital plate. **B.** Dorsal view of genital chamber and subgenital plate. **C.** Dorsal view of genital chamber and subgenital plate. Scale bars = 100 µm.

*Azteca* Forel, 1878 (Quate and Brown 2004). The previously published COI sequences plus the two new sequences from this study are the only published DNA barcodes for the genus *Alepis*, and no further molecular data are available for the remaining species. This fact becomes even more relevant when we know that *Alepis* is one of the most diverse Neotropical genera. All DNA barcodes of *A. viatrix* in BOLD cluster into two Barcode Index Numbers or BINs (Ratnasingham and Hebert 2013); the first BIN has one female specimen that differs 5.05–5.47% from all other specimens, and the second BIN comprises four specimens, including the male holotype, one male paratype, and the two females collected in Australia (with differences between 0.0% and 0.46%). Jaume-Schinkel et al. (2022) associated males and females on the basis of general morphology of both sexes and their co-occurrence at the same locality, as *A. viatrix* was the only species of *Alepis* present. We may provide arguments to justify the significant higher difference of the female paratype (5.05–5.47%) from the other sequenced specimens of *A. viatrix*, but they would be speculative. Despite this, we are confident that all of the specimens in the two BINs belong to the same species, based on the co-distribution of the specimens described, sequenced, and sex-associated by Jaume-Schinkel et al. (2022), the specimens sequenced herein, and the morphological comparison with the previously known female. Consequently, we believe that the relatively small uncorrected pairwise distance

between the two previously sequenced males and the herein sequenced females indicates that sex association in the genus *Alepis* can be done through DNA barcoding. However, as mentioned under Remarks, a broader representation with males and females of other *Alepis* species is necessary to understand the dissimilarity threshold for intra- and interspecific variability in this moth-fly genus.

Exochorionic structures in the eggs are species-specific and can help in the delimitation of species inside Psychodidae, and even delimit locality-specific populations (Almeida et al. 2004; Montes de Oca-Aguilar et al. 2017). We provide the first SEM images of the eggs for the genus *Alepis* and, as detailed in Figure 2A–C, the exochorion sculptures are quite distinctive with elongated hexagons along the long axis of the eggs. These egg structures may be a morphological characteristic to differentiate eggs of different species of the genus *Alepis*, but we do not have material of additional species for comparison. The only other Psychodinae exochorion description is of *Clogmia albipunctata* (Williston, 1893) (Rocha et al. 2011). Eggs of *C. albipunctata* present exochorion sculptures that continuous and discontinuous along the long axis, while eggs of *A. viatrix* form clear hexagonal-shaped sculpture along the long axis; thus, both species eggs can be easily differentiated by the shape of the exocorion sculpture.

Although species in the subfamily Psychodinae are not considered harmful to humans and/or domestic

animals, there is virtually no information on potential effects of the species on local native taxa. With the exception of a few species, there is no evidence of adult feeding in Psychodinae (Kvifte and Wagner 2017), and the ecological niche in which *A. viatrix* larvae develop is highly specific, i.e., water reservoirs inside bromeliad plants (phytotelmata). Bromeliads are native to the Neotropical Region (Breviglieri and Romero 2017), and some species can form phytotelmata, or water reservoirs, through the imbrication of the leaves; these water reservoirs are capable of maintaining small ecosystems with associated biota (Tsuda and Castellani 2016; Lopes-Filho et al. 2023). Introduced ornamental bromeliads (Kolicka et al. 2016; Wilke et al. 2018; Poniewozik et al. 2020) are implicated in facilitating establishment of viable populations in Florida of the non-native mosquito *Aedes albopictus* (Skuse, 1894) (Lounibos et al. 2003; Wilke et al. 2018), the vector for the transmission of several viral pathogens; thus, bromeliad phytotelmata are a good mechanism for passive invertebrate dispersal (Kolicka et al. 2016).

Australian specimens of *A. viatrix* were collected in an area where bromeliads are grown in residential gardens for decorative purposes. Moreover, records in the Atlas of Living Australia (ALA 2023) suggest bromeliads are widespread in the Brisbane area and evidently widely available for purchase from nurseries and garden centers (Howe pers. obs.). In addition, there are 28 records of bromeliads in the Brisbane area in the iNaturalist website (iNaturalist 2023), represented by six species, namely *Aechmea fasciata* (Lindl.) Baker, *Aechmea gamosepala* Wittm., *Ananas comosus* (L.) Merr., *Billbergia* sp. 1821; *Neoregelia* sp. 1934, and *Tillandsia usneoides* (L.) L. All species present on iNaturalist are non-native. It is noteworthy that, according to Arteaga et al. (2020: supplementary material 1), the two species present in the type locality of *A. viatrix* in the Azores are *Aechmea fasciata* and *Neoregelia carolinae* (Beer) L.B.Sm., which are also found in the surroundings of the new distributional records according to the iNaturalist records. Both species *Aechmea fasciata* and *Neoregelia carolinae* are naturally endemic to the Atlantic Forest in Brazil, primarily in the state of Rio de Janeiro (Martinelli et al. 2008). However, cultivated specimens may come from beyond their natural range, complicating the identification of the natural distribution of *A. viatrix*. Yet, it provides valuable insights into potential habitats for this species of moth fly.

The locality of the specimens described here (Yeronga State School; -27.519, 153.022) is 5 km from the nearest iNaturalist record of *A. fasciata* (-27.4935, 153.0439) and 3 km from the nearest record of *Neoregelia* sp. (-27.5135, 153.0039), and it is likely there are other bromeliads distributed in the area but not recorded on the Atlas of Living Australia or iNaturalist. In fact, 30% of the 80 student citizen scientists indicated they had bromeliads growing in their garden (distance to school = approximately 200 m to 7 km). Incidentally, an approximately 40 m<sup>2</sup> patch of false bromeliad plants (*Callisia*

*fragrans* (Lindl.) Woodson) was located (flowering) within 5 m of the Malaise trap at Yeronga State School (Howe pers. obs.). Although we do not have evidence that *A. viatrix* was imported to Australia through the commercialization of bromeliads, it is a likely scenario explaining the presence of this species and the gigantic gap between the new records, the known distribution of this species, and the hypothesized biogeographic origin of the genus *Alepis*.

Wagner and Hribar (2004) hypothesized that bromeliads containing larvae of *A. symmetrica* were imported from the Neotropics for decorative purposes, and, in the same way, bromeliads containing larvae of *A. vaga* were imported from Brazil to Sweden (Wagner and Svensson 2006). Similarly, Jaume-Schinkel et al. (2022) reported the presence of two bromeliad species, which were imported from Brazil, in the botanical garden in which the species *A. viatrix* was collected, and they argued these bromeliads are likely the cause of this *Alepis* species in the Azores. In summary, the international trade of bromeliads may be the cause of three *Alepis* species, hitchhikers which were unintentionally introduced to other countries.

Citizen-science projects have been successful in detecting and monitoring non-native species as well as providing new distributional data on native species (e.g. Gardiner et al. 2012; Johnson et al. 2020; Mengual and de Soto Molinari 2020; Feldman et al. 2021; Barahona-Segovia et al. 2022; Howard et al. 2022; Kvifte 2023; Jaume-Schinkel et al. in press). Whilst Insect Investigators did not primarily aim to act as a biosecurity surveillance program, the discovery of *Alepis* specimens in a Malaise trap in a primary school in a capital city is evidence of the diversity of scientific outcomes similar citizen-science projects can generate.

Finally, the involvement of youth in citizen-science projects can have a profound impact on species conservation efforts. By engaging young individuals in scientific research and data collection, they develop a deeper understanding and appreciation for the natural world around them. Citizen-science projects provide an opportunity for children to actively participate in real-world conservation efforts, fostering a sense of ownership and responsibility towards the environment. Through their involvement, children not only contribute valuable data that can aid in species monitoring and management, but also become advocates for biodiversity conservation in their communities. Additionally, participation in citizen science can spark curiosity and inspire future generations to pursue careers in science and conservation, ensuring the continuation of efforts to protect and preserve our planet's habitats and ecosystems.

## Acknowledgements

Insect Investigators received funding from the Australian government; it is led by the South Australian Museum and involves numerous partner organizations

including the University of Adelaide, Queensland Museum and the University of the Sunshine Coast. The Yeronga State School Malaise trap was run by Clare Trigell accompanied by teachers Katie McDermott, Ryan Henry, Josh Newby, Wendy Wooton, Kathy Lyons, and the enthusiastic participation of the remarkable young citizen scientists and students in year 4 (2022). Santiago Jaume-Schinkel's work on European Psychodidae is supported by the Bundesministerium für Bildung und Forschung, Berlin, Germany, the project "German Barcode of Life III: Dark Taxa" (FKZ 16LI1901A). We are thankful to Alessandre Pereira, Danilo Cordeiro, and Gunnar Kvifte for their comments that improved the final version of our publication.

## Author Contributions

Conceptualization: SJS. Data curation: XM, EPFJ, AGH, SJS. Formal analysis: XM, SJS. Funding acquisition: AGH, EPFJ. Investigation: SJS. Methodology: XM, SJS, AGH, EPFJ. Project administration: EPFJ, AGH. Resources: SJS, XM, EPFJ, AGH. Validation: XM, SJS. Visualization: SJS. Writing – original draft: SJS. Writing – review and editing: AGH, XM, EPFJ.

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## Supplemental Files

**Figure S1.** Examples of Year 4 (now 5 ) students' drawings of insects during an exploration of ecosystems and animal adaptions to their environment, including features and characteristics and how these suit particular environments.

**Figure S2.** Examples of Year 4 (now 5) students' drawings of insect adaptions to their environment and comparisons with introduced species, and negative effects of invasive species. Students were asked to hypothesize as to how *Alepis viatrix* was introduced to Australia.